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IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF :

Mitsuko ISHIHARA et al : EXAMINER: CHAKRABARTI, A.

SERIAL NO: 09/892,485 :

FILED: JUNE 28, 2001 : GROUP ART UNIT: 1634

FOR: METHOD FOR DETECTING ENDOCRINE DISRUPTING ACTION OF A TEST SUBSTANCE :

AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

In response to the Official Action mailed April 11, 2002, the Applicants respectfully request reconsideration of the rejections of record in view of the following amendments and remarks.

IN THE CLAIMS

Cancel Claim 5.

Amend the claims as follows:

1. (Amended) A method of detecting an endocrine disrupting action of a test substance, comprising:

(1) culturing a cell having a sensitivity to an endocrine hormone in a first culture system in which endocrine hormone and the test substance are present;

(2) determining the presence or absence of an endocrine disrupting action of the test substance by comparing a first gene expression pattern obtained from the cell of the first culture system with a second gene expression pattern expressed by a cell having a sensitivity to the endocrine hormone in the presence of the endocrine hormone, thereby detecting a gene specific to the first gene expression pattern; and

(3) detecting the presence or absence of a gene exhibiting a specific expression to a first gene expression pattern, thereby detecting the endocrine disrupting action of the test substance.

2. (Amended) A method of detecting an endocrine disrupting action of a test substance, comprising:

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(1) (a) culturing a cell having a sensitivity to an endocrine hormone in a first culture system in which the endocrine hormone and the test substance are present;

(b) culturing the cell having a sensitivity to the endocrine hormone in a third culture system in which the endocrine hormone is absent and the test substance is present; and

(2) determining the presence or absence of an endocrine disrupting action of the test substance by obtaining a first gene expression pattern obtained from the cell in the first culture system and a third gene expression pattern obtained from the cell in the third culture system, comparing the first gene expression pattern with the third gene expression pattern and a second expression pattern expressed by a cell having a sensitivity to the endocrine hormone in the presence of the endocrine hormone, thereby detecting a gene specific to the first gene expression pattern; and

(3) detecting the presence or absence of a gene exhibiting specific expression to the first gene expression pattern, thereby detecting the endocrine disrupting action of the test substance.

3. (Amended) A method of detecting an endocrine disrupting action of a test substance, comprising:

(1) (a) culturing a cell having a sensitivity to an endocrine hormone in a first culture system in which the endocrine hormone and the test substance are present;

(b) culturing the cell a having a sensitivity to the endocrine hormone in a third culture system in which the endocrine hormone is absent and the test substance is present;

(2) determining the presence or absence of an endocrine disrupting action of the test substance by comparing a first gene expression pattern obtained from the cell of the first culture system with a third gene expression pattern obtained from the cell in the third culture system, thereby detecting a gene specific to the first gene expression pattern; and

(3) detecting the presence or absence of a gene exhibiting specific expression to the first gene expression pattern, thereby detecting the endocrine disrupting action of the test substance.

4. (Amended) A method of detecting an endocrine disrupting action of a test substance, comprising:

(1) (a) culturing a cell having a sensitivity to an endocrine hormone in a first culture system in which the endocrine hormone and the test substance are present;

(b) culturing the cell having a sensitivity to the endocrine hormone in a second culture system in which the endocrine hormone is present and the test substance is absent; and

(c) culturing the cell having a sensitivity to the endocrine hormone in a third culture system in which the endocrine hormone is absent and the test substance is present; and

(2) determining the presence or absence of an endocrine disrupting action of the test substance by comparing a first gene expression pattern obtained from the cell in the first culture system with a second gene expression pattern obtained from the cell in the second culture system and the third gene expression pattern obtained from the cell in the third culture system, thereby detecting a gene specific to the first gene expression pattern; and

(3) detecting the presence or absence of a gene exhibiting specific expression to the first gene expression pattern, thereby detecting the endocrine disrupting action of the test substance.

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6. (Amended) The method according to claim 1, wherein said endocrine hormone is triiodothyronine and said cell having a sensitivity to the endocrine hormone is Neuro2a.

7. (Amended) The method according to claim 1, wherein comparison of the gene expression patterns are made by comparing bands obtained by subjecting a gene group contained in each of the gene expression patterns to electrophoretic separation.

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8. (Amended) The method of detecting an endocrine disrupting action of a test substance according to claim 1, comprising

(a) recovering RNAs from each of the culture systems of (1);

(b) subjecting the RNAs recovered in the step (a) to reverse transcription;

(c) amplifying reverse transcription products obtained in (b) by PCR; and

(d) subjecting PCR products obtained in step (c) to electrophoresis, comparing electrophoretic patterns of the bands obtained, thereby detecting a band specific to the first gene expression pattern.

9. (Amended) The method according to claim 1, wherein said gene expression patterns are compared by hybridizing gene groups contained in each of the gene expression patterns with each other, and subtracting unhybridized genes.

10. (Amended) A method of detecting an endocrine disrupting action of a test substance, comprising:

(1) determining the presence or absence of an endocrine disrupting action of the test substance by

(a) culturing a cell having a sensitivity to an endocrine hormone in a first culture system in which the endocrine hormone and the test substance are present;

(b) culturing a cell having a sensitivity to the endocrine hormone in a second culture system in which the endocrine hormone is present and the test substance is absent;

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(c) culturing a cell having a sensitivity to the endocrine hormone in a third culture system in which the endocrine hormone is absent and the test substance is present; and

(d) culturing a cell having a sensitivity to the endocrine hormone in a fourth culture system in which both the endocrine hormone and the test substance are absent;

(2) (a) isolating RNA from the first culture system and preparing a first cDNA based on the RNA;

(b) isolating a second RNA from the second culture system;

(c) isolating a third RNA from the third culture system; and

(d) isolating RNA from the fourth culture system and preparing a fourth cDNA based on the RNA;

(3) (a) hybridizing the first cDNA and the second RNA and recovering unhybridized cDNA;

(b) hybridizing the third RNA and the fourth cDNA, recovering unhybridized RNA; and

(4) hybridizing the cDNA obtained in (a) of (3) and the RNA obtained in (b) of (3); and recovering unhybridized RNA, thereby detecting a gene specific to an endocrine disrupting action; and

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(5) detecting a specific gene to the endocrine disrupting action, thereby detecting the endocrine disrupting action of the test substance.

14. (Amended) A method of detecting an endocrine disrupting action of a test substance, comprising:

(1) culturing a cell having a sensitivity to an endocrine hormone in a first culture system with the endocrine hormone and the test substance;

(2) determining the presence or absence of an endocrine disrupting action of the test substance by comparing a first glycoprotein pattern obtained from the cell of the first culture system with a second glycoprotein pattern expressed by a cell having a sensitivity to the endocrine hormone in the presence of the endocrine hormone, thereby detecting a glycoprotein specific to the first glycoprotein pattern; and

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(3) determining the presence and absence of a glycoprotein exhibiting specific expression to the first glycoprotein expression pattern, thereby detecting the endocrine disrupting action of the test substance.

15. (Amended) A method of detecting an endocrine disrupting action of a test substance, comprising:

(1) (a) culturing a cell having a sensitivity to an endocrine hormone in a first culture system with the endocrine hormone and the test substance;

(b) culturing a cell having a sensitivity to the endocrine hormone in a third culture system in which the endocrine hormone is absent and the test substance is present; and

(2) determining the presence or absence of endocrine disrupting action of the test substance by

obtaining a first glycoprotein pattern obtained from the cell of the first culture system and a third glycoprotein pattern obtained form the cell of the third culture system, and

comparing the first glycoprotein pattern with the third glycoprotein pattern and a second glycoprotein pattern obtained from a cell having a sensitivity to the endocrine hormone in the presence of the endocrine hormone, thereby detecting a glycoprotein specific to the first glycoprotein pattern; and

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(3) determining the presence and absence of a glycoprotein exhibiting specific expression to the first glycoprotein expression pattern, thereby detecting the endocrine disrupting action of the test substance.

16. (Amended) A method of detecting an endocrine disrupting action of a test substance, comprising:

(1) (a) culturing a cell having a sensitivity to an endocrine hormone in a first culture system in which the endocrine hormone and the test substance are present;

(b) culturing a cell having a sensitivity to the endocrine hormone in a third culture system in which the endocrine hormone is absent and the test substance is present; and

(2) determining the presence or absence of endocrine disrupting action of the test substance by comparing the first glycoprotein pattern obtained from the cell of the first

culture system with the third glycoprotein pattern obtained from the cell of the third culture system, thereby detecting a glycoprotein specific to the first glycoprotein pattern; and

(3) determining the presence and absence of a glycoprotein exhibiting specific expression to the first glycoprotein expression pattern, thereby detecting the endocrine disrupting action of the test substance.

17. (Amended) A method of detecting an endocrine disrupting action of a test substance, comprising:

(1) (a) culturing a cell having a sensitivity to an endocrine hormone in a first culture system in which the endocrine hormone and the test substance are present;

(b) culturing a cell having a sensitivity to the endocrine hormone in a second culture system in which the endocrine hormone is present and the test substance is absent; and

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(c) culturing a cell having a sensitivity to the endocrine hormone in a third culture system in which the endocrine hormone is absent and the test substance is present;

(2) determining the presence or absence of an endocrine disrupting action of the test substance by comparing first, second and third glycoprotein patterns obtained respectively from the cells of the first, second, third culture systems, thereby detecting a glycoprotein specific to the first glycoprotein pattern; and

(3) determining the presence and absence of a glycoprotein exhibiting specific expression to the first glycoprotein expression pattern, thereby detecting the endocrine disrupting action of the test substance.

18. (Amended) The method according to claim 14, wherein said endocrine hormone is selected from the group consisting of estrogen, estradiol, progesterone, androgen, testosterone, androsterone, cortisol, aldosterone, corticosterone, cortison, triiodothyronine,

and thyroxine; and said cell having a sensitivity to the endocrine disrupting action is selected from the group consisting of Neuro2a, S-20Y, MCF7, TM3, TM4 and 15P-1.

19. (Amended) The method according to claim 14, wherein said endocrine hormone is triiodothyronine and said cell having a sensitivity to the endocrine hormone is Neuro2a.

20. (Amended) The method according to claim 14, wherein the glycoprotein specific to the first culture system is performed by a method comprising:

(a) extracting proteins biosynthesized by a cell contained in each of culture systems of (1);

(b) recovering a glycoprotein by binding a substance for specifically binding to a polysaccharide chain contained in the proteins extracted in (a) to the polysaccharide chain;

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(c) recovering a protein contained in the glycoprotein by cutting off the polysaccharide chain; subjecting the protein to electrophoresis, thereby obtaining a glycoprotein pattern for each of the culture systems; and

(d) comparing glycoprotein patterns to each other.

Please add new Claims 24-28 as follows:

24. (New) A method of detecting an endocrine disrupting action of a test substance, comprising:

(1) culturing a cell having a sensitivity to an endocrine hormone in a first culture system in which the endocrine hormone and the test substance are present, wherein said endocrine hormone is selected from the group consisting of a female hormone, male hormone, adrenal cortex hormone, and an amino acid derivative hormone;

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(2) determining the presence or absence of an endocrine disrupting action of the test substance by comparing a first gene expression pattern obtained from the cell of the first

culture system with a second gene expression pattern expressed by a cell having a sensitivity to the endocrine hormone in the presence of the endocrine hormone, thereby detecting a gene specific to the first gene expression pattern; and

(3) determining the presence and absence of a glycoprotein exhibiting specific expression to the first glycoprotein expression pattern, thereby detecting the endocrine disrupting action of the test substance.

25. (New) The method according to Claim 24, wherein said cell having a sensitivity to the endocrine disrupting action is selected from the group consisting of Neuro2a, S-20Y, MCF7, TM3, TM4 and 15P-1.

26 (New) The method according to Claim 24, wherein comparison of the gene expression patterns is made by comparing bands obtained by subjecting a gene group contained in each of said gene expression patterns to an electrophoretic separation.

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27. (New) The method of detecting an endocrine disrupting action of a test substance according to Claim 24, comprising:

(a) recovering RNAs from each of the culture systems of (1);

(b) subjecting the RNAs recovered in the step (a) to reverse transcription;

(c) amplifying reverse transcription products obtained in (b) by PCR; and

(d) subjecting PCR products obtained in step (c) to electrophoresis, comparing the electrophoretic patterns of bands obtained, thereby detecting a band specific to a first gene expression pattern.

28. (New) The method according to Claim 24, wherein said gene expression patterns are compared by hybridizing gene groups contained in each of the gene expression patterns with each other, and subtracting unhybridized genes.

REMARKS

Claims 1-4 and 6-28 are pending. Claims 1-4, 6-10 and 15-25 are active. Like the elected claims, Claim 14 is also directed to a method of detecting, and not to a product, therefore, the Applicants respectfully submit that this claim should be rejoined to the elected group of claims.. To help further clarify the invention, the Applicants attach herewith a Declaration further describing and elaborating on the advantages of the invention. Also, as suggested by the Examiner, Claims 1-4, 9, and 14-17 have been amended to recite a final detecting step that relates back to the preambles of these claims. Support is found in the original claim language and on pages 2-7 of the specification. Support for new Claim 24 is found in original Claim 5. New Claims 25-28 depend from Claim 24 and find support in original Claim 5 and Claims 6-9. Accordingly, the Applicants do not believe that any new matter has been added.

RESTRICTION

Applicants hereby affirm their prior election with traverse of Group I, Claims 1-10 and 15-20 and respectfully request that Claim 14 be rejoined to the elected claims. Claim 14 is directed to a method of detecting, and not to a polynucleotide, and should therefore be grouped with Claims 1-10 and 15-20. Rejoinder is therefore respectfully requested.

REJECTION--35 U.S.C. 112, SECOND PARAGRAPH

Claims 1-10 and 15-20 were rejected under 35 U.S.C. 112, second paragraph, as being indefinite. The bodies of Claims 1-4, 10 and 15-17 have been amended to relate back to the preamble and Claim 20 has been amended to delete the phrase "capable of". Accordingly, these rejections may now be withdrawn.

REJECTION--35 U.S.C. 102(e)

Claims 1-4 were rejected under 35 U.S.C. 102(e) as being anticipated by Lonial et al., U.S. Patent 6,001,560. Lonial does not anticipate the present invention, because it is directed to a simple promoter gene assay for determining whether a compound exerts IFN-γ activity, and not to a method for determining whether a test substance disrupts the activity of an endocrine hormone present in the medium in which a cell is cultured. Moreover, the promoter gene assay of Lonial does not detect an endocrine disrupting action of a test substance by comparing the gene expression patterns of cells exposed to the test substance in the presence of the endocrine hormone, and those not exposed to it.

On the other hand, the present invention detects the endocrine disruption activity of a test substance based on a differential display method. This method measures differences in gene expression in the presence of, and absence of, a particular test substance, e.g., an endocrine hormone disrupting substance, such as dioxin. The effect of the test substance is determined by comparing the expression patterns of the cells exposed to the test substance, with the expression patterns of cells not exposed to the test substance. By culturing the cells in the presence of the endocrine hormone, the disruptive effects of the putative test substance can be accurately determined under more natural conditions, e.g., in the presence of the natural endocrine hormone to which hormonally-sensitive cells in an animal would normally be exposed. The advantages and distinguishing features of the present invention are further described in the attached Declaration.

On the other hand, Lonial is directed to a promoter gene assay method for determining whether a substance exhibits an IFN-γ like activity based on expression of a reporter gene (e.g. a growth hormone gene) that has been constructed and transformed into a

reporter cell line. Such a method uses a specific cell for observing a specific action (i.e. IFN-γ action) and does not detect an unspecified action such as an endocrine disrupting action. Lonial does not describe the method of the present invention that comprises detecting an endocrine disrupting action of a test substance by (1) culturing a cell sensitive to an endocrine hormone in the presence of the endocrine hormone and test substance and (2) comparing the gene expression patterns of cells exposed to the test substance and the cells not exposed to the test substance.

Lonial does not disclose culturing a cell sensitive to an endocrine hormone in the presence of the endocrine hormone and a test substance, or disclose comparing the gene expression patterns of cells exposed to the test substance, with those not exposed to the test substance. For instance, Claim 12 of Lonial is directed to contacting a transformed cell line with a sample suspected to containing a human IFN-γ agonist and measuring the level of expression of a reporter gene (e.g., growth hormone). Lonial do not disclose (1) culturing a cell sensitive to an endocrine hormone in the presence of the endocrine hormone and a test substance, and (2) comparing the gene expression patterns of cells exposed to the test substance and the cells not exposed to the test substance.

Unlike the present invention, Lonial is not concerned with whether a substance (e.g., dioxin) exhibits an endocrine hormone disrupting action, only with whether a particular compound exhibits an IFN-γ like activity as measured by the expression of a reporter gene, such as a growth hormone gene that has been transformed into a cell line. Moreover, the method of Lonial would be ineffective, if the cell lines which express growth hormone reporter gene were grown in the presence of growth hormone, as the level of secreted growth hormone protein from the reporter gene could not be accurately measured.

Accordingly, the Applicants respectfully request that this rejection now be withdrawn.

REJECTION--35 U.S.C. 103

Claims 1-4, 7, 9 and 15-17 were rejected under 35 U.S.C. 103(a) as being unpatentable over Lonial et al., U.S. Patent 6,001,560, in view of Gilles et al., U.S. Patent 4,663,281. Lonial and Gilles do not render the invention obvious, as neither document suggests a method for detecting an endocrine disrupting action of a test substance by (1) culturing a cell sensitive to an endocrine hormone in the presence of the endocrine hormone and test substance and (2) comparing the gene expression patterns of cells exposed to the test substance and the cells not exposed to the test substance.

As discussed above, Lonial is directed to determining whether a substance exhibits an IFN-γ like activity based on expression of a reporter gene (e.g. a growth hormone gene) and is not concerned with whether a substance (e.g., dioxin) exhibits an endocrine disrupting action.

Gilles is directed to a method for enhancing the production of proteins in eukaryotic cells and does not describe a method for testing whether a substance (e.g., dioxin) exhibits an endocrine disrupting action. Fig. 2 of Gilles shows an autoradiogram of radiolabelled proteins produced by cell lines transfected with different plasmids; Fig. 7 shows a Southern blot comparing the DNA's of cells transfected with different plasmids; and Fig. 8 shows a Northern blot comparing the RNA's of cells transfected with different plasmids. However, none of these assays were performed by (1) culturing a cell sensitive to an endocrine hormone in the presence of the endocrine hormone and putative endocrine disrupting test

substance and (2) comparing the gene expression patterns of cells exposed to the test substance and the cells not exposed to the test substance.

Moreover, none of the cited prior art suggests the advantages of the present invention which are shown in the attached Declaration. As shown in Table 3 of the Declaration, the present invention makes it possible to detect the difference in the expression numerous different genes associated with an endocrine disrupting action, when such gene expression was not be detected by a comparable conventional method (e.g., a method that did not culture cells in the presence of an endocrine hormone). Graph 1 of the Declaration shows the effects of dioxin on five different types of genes specifically identified by the method of the present invention in the presence of the endocrine hormone T3. However, conventional methods that did not culture cells in the presence of the T3 hormone, did not identify the ability of dioxin to disrupt these genes or identify the sensitivity of these genes to dioxin.

Accordingly, as the prior art does not disclose or suggest the invention, or the superior ability of the invention for identifying particular endocrine disrupting compounds or genes responsive to such disruption, the Applicants respectfully request that this rejection be withdrawn.

REJECTION--35 U.S.C. 103

Claims 1-4, 8 and 10 were rejected under 35 U.S.C. 103(a) as being unpatentable over Lonial et al., U.S. Patent 6,001,560, in view of Pearson et al, U.S. Patent 5,916,779. Lonial and Pearson do not render the invention obvious, as neither document suggests a method for detecting an endocrine disrupting action of a test substance by (1) culturing a cell sensitive to an endocrine hormone in the presence of the endocrine hormone and test substance and (2) comparing the gene expression patterns of cells exposed to the test substance and the cells

not exposed to the test substance. Lonial has been discussed above. Pearson is cited for its teachings of RT PCR on RNA recovered from a cell, however, it does not remedy the deficiencies of Lonial, because it does not suggest detecting endocrine disrupting substances by a differential display method in the presence of an endocrine hormone. Moreover, the cited prior art does not suggest the superior sensitivity of the present invention for identifying particular endocrine disrupting compounds or genes responsive to such disruption. Accordingly, the Applicants respectfully request that this rejection be withdrawn.

REJECTION--35 U.S.C. 103

Claims 1-6 were rejected under 35 U.S.C. 103(a) as being unpatentable over Lonial et al., U.S. Patent 6,001,560, in view of Comoglio et al., U.S. Patent 6,030,949, further in view of Cubicciotti, U.S. Patent 6,287,765 B1. Lonial, in view of Comoglio and Cubicciotti do not render the invention obvious, as these documents do not suggest a method for detecting an endocrine disrupting action of a test substance by (1) culturing a cell sensitive to an endocrine hormone in the presence of the endocrine hormone and test substance and (2) comparing the gene expression patterns of cells exposed to the test substance and the cells not exposed to the test substance. Comoglio, Examples 2 and 3, is cited for its teaching of Neuro2a cells and Cubicciotti for its teaching of the hormone triiodothyronine. However, neither of these documents discloses the method of the present invention involving (1) culturing a cell sensitive to an endocrine hormone in the presence of the endocrine hormone and test substance and (2) comparing the differential gene expression patterns of cells exposed to the test substance and the cells not exposed to the test substance. Thus, these documents do not remedy the deficiencies in Lonial. Moreover, the cited prior art does not suggest the superior ability of the present invention for identifying particular endocrine

disrupting compounds or genes responsive to such disruption. Accordingly, the Applicants respectfully request that this rejection be withdrawn.

REJECTION--35 U.S.C. 103

Claims 1-6 were rejected under 35 U.S.C. 103(a) as being unpatentable over Lonial et al., U.S. Patent 6,001,560, in view of Gilles et al., U.S. Patent 4,663,281, further in view of Comoglio et al., U.S. Patent 6,030,949, further in view of Cubicciotti, U.S. Patent 6,287,765 B1. As discussed above, none of the cited documents disclose or suggest a method of (1) culturing a cell sensitive to an endocrine hormone in the presence of the endocrine hormone and test substance and (2) comparing the differential gene expression patterns of cells exposed to the test substance and the cells not exposed to the test substance. Moreover, the cited prior art does not suggest the superior ability of the present invention for identifying particular endocrine disrupting compounds or genes responsive to such disruption. Accordingly, this rejection may now also be withdrawn.

REJECTION--35 U.S.C. 103

Claims 1-6 were rejected under 35 U.S.C. 103(a) as being unpatentable over Lonial et al., U.S. Patent 6,001,560, in view of Gilles et al., U.S. Patent 4,663,281 further in view of Makari, U.S. Patent 4,752,471. As discussed above, Lonial and Gilles do not disclose or suggest a method of (1) culturing a cell sensitive to an endocrine hormone in the presence of the endocrine hormone and test substance and (2) comparing the gene expression patterns of cells exposed to the test substance and the cells not exposed to the test substance. Makari, Claim 5, is cited for its teaching of recovering a glycoprotein by cutting off the polysaccharide chain. However, Makari does not remedy the primary deficiencies of Lonial or Gilles, as discussed above. Moreover, the cited prior art does not suggest the superior

sensitivity of the present invention for identifying particular endocrine disrupting compounds or genes responsive to such disruption. Accordingly, this rejection may now also be withdrawn.

CONCLUSION

In view of the above amendments and remarks, the Applicants respectfully submit that this application is now in condition for allowance. Early notification to that effect is earnestly solicited.

Respectfully submitted,

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